

Original Article

Metabolic and Hormonal Determinants of Glomerular Filtration Rate and Renal Hemodynamics in Severely Obese Individuals

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Key Words

Morbid obesity · Glomerular filtration rate · Renal plasma flow · Augmentation index

Abstract

Objective: Renal function is often compromised in severe obesity. A true measurement of glomerular filtration rate (GFR) is unusual, and how estimation formulae (EstForm) perform in such individuals is unclear. We characterized renal function and hemodynamics in severely obese individuals, assessing the reliability of EstForm. **Methods:** We measured GFR (mGFR) by iothexol plasma clearance, renal plasma flow (RPF) by ¹²³I-ortho-iodo-hippurate, basal and stimulated vascular renal indices, endothelium-dependent and -independent vasodilation using flow-mediated dilation (FMD) as well as metabolic and hormonal profile in morbid, otherwise healthy, obese subjects. **Results:** Compared with mGFR, the better performing EstForm was CKD-EPI (5.3 ml/min/1.73 m² bias by Bland-Altman analysis). mGFR was directly related with RPF, total and incremental glucose AUC, and inversely with PTH and h8 cortisol. Patients with mGFR below the median shown significantly higher PTH and lower vitamin D3. Basal or dynamic renal resistive index, FMD, pulse wave velocity were not related with mGFR. In an adjusted regression model, renal diameter and plasma flow remained related with mGFR (R² = 0.67), accounting for 15% and 21% of mGFR variance, respectively. **Conclusions:** CKD-EPI formula should be preferred in morbid obesity; glucose increments during oral glucose tolerance test correlate with hyperfiltration; RPF and diameter are independent determinants of mGFR; slightly high PTH values, frequent in obesity, might influence mGFR.

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Introduction

Obesity is an independent risk factor and a powerful predictor of chronic kidney disease (CKD) and end-stage renal disease [1–3]. Among the mechanisms underlying obesity-associated renal pathology, abnormalities of sodium sensitivity, insulin resistance, and hyperaldosteronism are recognized [4, 5].

A fast glomerular filtration rate (GFR) decline, even remaining above the usual threshold marking the definition of CKD (60 ml/min/1.73 m²), is a powerful determinant of cardiovascular (CV) disease in the general population and in high-risk individuals [6, 7]. Therefore, an accurate assessment of renal function is strongly recommended in the clinical practice to better define the individual renal and CV risk profile.

Renal injury associated with obesity shows a high prevalence of albuminuria and inflammation-driven glomerular lesions [8, 9]. Moreover, a relative glomerular hyperfiltration is frequent [10, 11], and obesity-related glomerulopathy itself can be considered a maladaptive response to hyperfiltration and albuminuria [12]. Hyperglycemia is a major determinant of hyperfiltration in diabetes [13], while data obtained in morbid obesity are scanty.

In the clinical practice, a range of bedside tools, based on mathematical formulae including age, gender, serum creatinine and sometimes body weight, are used to estimate GFR. Though each of these formulae has its own limitations, they can be reliably used in the normal population, with a reported better accuracy of the CKD-EPI than the MDRD study equation for higher GFR levels [14, 15]. Given the high prevalence of obesity in the western countries, an obvious question concerns the reliability of these formulae in obese patients [16] and whether or not they require correction for body weight confounders like total and lean weight and body surface area [17, 18]. Studies validating these formulae in morbidly obese individuals are lacking.

Doppler ultrasonography, routinely used to characterize CKD, allows to identify macro- and microvascular abnormalities, with intra-parenchymal arterial waveform offering information on arteriosclerosis and interstitial fibrosis [19]. Among ultrasonographic indicators of renal hemodynamics, renal resistive index (RI) has been related with BMI [20], but no study have so far related RI and its variation to a vasodilatory stimulus to the filtration function in severely obese individuals. In this view, it is interesting to assess by exploring the relationship between GFR and renal vascular reactivity whether or not the true measurement can be reliably replaced by the estimation value. Therefore, we designed a protocol to compare estimated GFR (eGFR) and ultrasonographic assessment of renal vasculature with true measurements of renal filtration and flow in a group of severely obese individuals, relating these observations with their metabolic and hormonal patterns.

Patients and Methods

Patients

50 morbidly obese individuals (BMI > 40 kg/m²) participating in a clinical program to evaluate their eligibility for bariatric surgery were recruited in the years 2013–2014 in the section of Metabolic Medicine in our hospital. Exclusion criteria were age > 60 years, diabetes (excluded by a standard oral glucose tolerance test (OGTT) performed 1 week before the study day), essential hypertension, systemic inflammatory diseases, serum creatinine above the normal range, and presence of micro- or macroalbuminuria (excluded by nephelometric analysis on a 24-hour urine collection performed 2 weeks before the study day). The protocol was approved by the Ethics Committee of the University of Pisa (n.3463/2011). All participants signed an informed consent.

After an overnight fast, patients underwent a clinical examination at the study day. Blood pressure (BP) was measured in the supine position by an oscillometric sphygmomanometer after a 10-min rest using an appropriately sized cuff. Three consecutive measurements were taken, averaging the last two. Blood samples were collected to determine glucose, cholesterol, triglycerides, HDL-cholesterol, creatinine, uric acid, and the hormonal profile (TSH, GH, ACTH, cortisol, PTH).

Estimation and Evaluation of Glomerular Function

GFR was measured by the iothexol plasma clearance (mGFR) method. Briefly, catheters were inserted into antecubital veins for injection of the filtration marker and blood sampling. Blood was taken for background measurement and iothexol standard preparation; a 5 ml i.v. bolus of iothexol (Omnipaque 300; iothexol 647 mg/ml, corresponding to 300 mg/ml of iodine; Nycomed, Milano, Italy) was then injected within 30 s followed by saline solution 10 ml, and blood samples were drawn at 5, 15, 60, 90, 120, 150, 180, 210, 240, and 300 min. After centrifugation at room temperature (3,000 rpm for 10 min), and plasma separation, iothexol concentrations were determined in duplicate by HPLC (Perkin Elmer, Waltham, MA, USA). Briefly, plasma samples were deproteinized with 4 vol 5% perchloric acid and centrifuged twice (13,000 rpm for 5 min). 20 µl of supernatant were assayed in duplicate through a Pico Tag Column (Waters, Milford, MA, USA), inserting a pre-column between the injector and the analytical column. The detector was set at 254 nm, using a mixture of deionized water and acetonitrile (96:4, pH 2.5 with phosphoric acid) as mobile phase, pumped at a rate of 1.1 ml/min. Iothexol elution from the column was observed after 5 min. As expected, two peaks reflecting its isomeric forms in the pharmacological preparation were evident, and the second peak was used for plasma clearance calculation. Iothexol concentrations were determined by comparing the peak height of each sample with that of the standard curve, obtained by adding known amounts of iothexol to the zero-time plasma sample.

DIMSUM, an expert system for multi-exponential model discrimination, was employed to fit plasma disappearance curve over time by a 2nd-order model for iothexol concentrations. Elimination constants (α_1 and α_2) and intercepts (A_1 and A_2) were used to calculate the area under the disappearance curve (AUC): $AUC = (A_1/\alpha_1) + (A_2/\alpha_2)$. Plasma iothexol clearance was calculated as the administered dose (double weighing of the injection syringe) divided by AUC.

Values obtained by the above reported technique were compared with the eGFR calculated by the following equations: Cockcroft-Gault (adjusted by body surface area calculated according to Mosteller formula – (height (cm) × weight (kg) / 3,600)^{0.5} –, MDRD, and CKD-EPI.

Measurement of Renal Plasma Flow

The whole procedure for measuring the effective renal plasma flow (RPF) was performed according to published guidelines [21]. Two syringes (dose and standard) were prepared with 7 MBq of ¹²³I-ortho-iodo-hippurate (¹²³I-OIH Hippuran; Covidien, Dublin, Ireland) and assayed in the dose calibrator to determine the actual administered activity. Radiotracer was intravenously administered as bolus; another i.v. line in the opposite arm served to withdraw blood samples. To obtain a complete plasma curve, 5 ml blood was drawn at 5, 10, 15, 20, 30, 40, and 60 min post-injection in anticoagulant citric dextrose tubes. Plasma was centrifuged and two 1-ml aliquots were withdrawn for counting. Standard was diluted till 1,000 ml with water, and three count vials were obtained. Plasma and standard were counted by an AtomLab 950 (Biodex Medical Systems Inc., Shirley, NY, USA). Counts (cpm/ml) from each plasma aliquot and for the three separate standards were averaged.

Injected dose was calculated by multiplying the final value of the standard corrected for the dilution factor by the ratio between activity (MBq) of the injected dose and standard. Multiple blood sample clearance curves were fitted with a two-exponential model using the curve fitting tool of the software MatLab 7.9.0 (The Mathworks™), and RPF was calculated as follows:

$$RPF \text{ (ml/min)} = \text{injected dose} / (A/\alpha + B/\beta) \quad (1),$$

where A and B are the y-axis intercepts of each exponential component and α , and β are the respective slopes.

Baseline and Dynamic Vascular Renal Indices

RI was obtained in resting conditions and 5 min after 25 µg of sublingual glyceryl trinitrate (GTN). A complete renal scan, including measurement of longitudinal renal diameter, was performed by a single trained operator (R.M.B.) with an ultrasound machine (MyLab 25; ESAOTE, Florence, Italy) equipped with a high-resolution multifrequency Convex probe (2.5–4.5 MHz). Three velocimetric measurements of the interlobar renal arteries in both kidneys adjacent to medullary pyramids were obtained by a translumbar approach. RI was calculated as follows:

$$RI = (\text{systolic peak velocity} - \text{end diastolic velocity}) / \text{systolic peak velocity} \quad (2).$$

Dynamic resistive index was calculated as percent changes from baseline in response to GTN.

Endothelium-Dependent and -Independent Vasodilation in the Brachial Artery

Endothelium-dependent vasodilation of the brachial artery was assessed by the flow-mediated dilation (FMD) technique, as previously described [22]. A cuff was positioned around the right forearm; the right brachial artery was located and scanned longitudinally 5–10 cm above the elbow using a 10 MHz linear array transducer (MyLab 25), maintaining the probe in the same position by a stereotactic clamp. The cuff was then inflated at 300 ± 30 mm Hg and deflated after 5 min; response to reactive hyperemia was observed in the following 4 min. FMD was calculated as the maximal percent increase in diameter above baseline (mean of 1-min recordings). Brachial artery diameter was measured by a real-time computerized edge detection system, allowing simultaneous continuous measurements of brachial artery diameter and flow velocity (Cardiovascular Suite; Quipu srl, Pisa, Italy).

Arterial Tonometry

Carotid-femoral pulse wave velocity (PWV) was assessed by arterial tonometry (SphygmoCor, AtCor Medical, West Ryde, Australia), according to international recommendations [23]. PWV was calculated as the ratio of the direct femoral-carotid distance, multiplied by 0.8 and wave transit time. Radial waveform was also acquired to obtain aortic pressure waveform by means of a validated transfer function on three successive measurements. Augmented pressure (AP) was calculated as difference between the second and the first systolic peak, and augmentation index (AIx) as the ratio between augmented pressure and pulse pressure, normalized at 75 bpm heart rate.

Analytical Determinations

Plasma glucose was measured by the hexokinase method. Values from OGTT were used to evaluate the AUC and the incremental area under the curve (IncrAUC). Cholesterol and triglycerides were determined by enzymatic colorimetric assays; HDL cholesterol was measured enzymatically. LDL cholesterol was calculated by Friedewald formula. Blood cell count and liver enzymes were measured by routine methods. The uricase/PAP method and a modified Jaffé method were used for uric acid and creatinine determination, respectively.

Serum FT4, FT3, TSH, PTH and 25(OH)vitamin D were measured by immunoradiometric assays (DiaSorin, Saluggia, Italy). Cortisol was assessed by chemiluminescent microparticle immunoassay technology (ARCHITECT®, Abbott Laboratories, Chicago, IL, USA); ACTH via Immunoradiometric Assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). GH was measured by automated chemiluminescent GH assay (DiaSorin); its sensitivity was 0.05 µg/l; intra- and inter-assay CV were 4.1 and 7.3% respectively.

Statistical Analysis

All values are expressed as mean \pm SD, unless otherwise specified. mGFR obtained by the iothexol technique was compared with eGFR calculated by Cockcroft-Gault, MDRD, and CKD-EPI equations using Pearson's coefficient (R); a Bland-Altman analysis was also performed [24] and bias calculated. Bland-Altman plots were obtained by Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). Comparisons were performed using the nonparametric Mann-Whitney U-test or the unpaired t-test, for variables with non-normal or normal distribution. Clinical, hormonal, and hemodynamic correlates of mGFR were assessed using Spearman's rank correlation and multiple linear regression analysis. A p value < 0.05 was considered significant. Analyses were performed using NCSS8 software (NCSS, Kaysville, UT, USA).

Results

Comparison between mGFR and eGFR

Clinical characteristics of the study participants are shown in table 1. All patients had normal fasting glucose and BP values; female gender prevailed; 16% of the participants were smokers.

Table 2 reports renal functional parameters and the hormonal profile of the study participants. mGFR did not significantly differ when compared to that obtained in a group of 10 healthy individuals with normal BMI that served as internal reference (102.33 ± 14.96 vs. 105.95 ± 13.36 ml/min/1.73 m²). When compared with mGFR, the estimation formula with the best performance was the CKD-EPI (R = 0.49, p = 0.0003); this was also true in subjects

Table 1. Clinical characteristics of obese individuals. Data are expressed as mean \pm SD, median (IQ range) or number and %.

Age, years	44.2 \pm 11.8
Gender (M/F)	19/31
Smokers (n)	8
BMI, kg/m ²	45.98 \pm 6.98
Systolic blood pressure, mm Hg	136.1 \pm 5.3
Diastolic blood pressure, mm Hg	81.6 \pm 5.4
Albumin excretion rate, mg/24 h	5.6 (4.0–8.2)
Fasting blood glucose, mg/dl	95.2 \pm 18.4
Total cholesterol, mg/dl	185.1 \pm 30.6
HDL cholesterol, mg/dl	44.9 \pm 11.1
LDL cholesterol, mg/dl	114.1 \pm 24.8
Triglycerides, mg/dl	153.4 \pm 80.7
Lp(a), mg/dl	16.2 \pm 17.6
BUN, mg/dl	14.8 \pm 2.9
Uric acid, mg/dl	5.77 \pm 1.49
AST, IU/l	27.8 \pm 13.8
ALT, IU/l	34.8 \pm 17.3
γ GT, IU/l	30.4 \pm 16.7
Fibrinogen, mg/dl	344.7 \pm 52.8
RBC (n/mm ³)	4.71 \pm 0.37
Hb, g/dl	13.4 \pm 1.4
WBC (n/mm ³)	7.19 \pm 1.86
Platelets (n/mm ³)	262 \pm 50

Table 2. Measured and estimated (according to MDRD, CKD-EPI and Cockcroft-Gault algorithms) glomerular filtration rate and hormone pattern in the whole study cohort (n = 50) and divided into above (n = 26) and below (n = 24) the median mGFR value (104.20 ml/min/1.73 m²)^a

Parameters (normal range)	All	Above	Below	p value
True GFR	102.33 \pm 14.96	113.91 \pm 5.61	89.78 \pm 11.26	<0.001
eGFR (MDRD)	91.05 \pm 20.55	96.71 \pm 15.60	84.91 \pm 23.65	0.041
eGFR (CKD-EPI)	97.05 \pm 17.08	102.06 \pm 12.87	91.63 \pm 19.55	0.030
eGFR (Cockcroft-Gault)	140.07 \pm 36.20	148.71 \pm 30.59	130.70 \pm 39.98	0.079
GH, ng/ml (0.03–0.97)	0.14 \pm 0.10	0.13 \pm 0.10	0.15 \pm 0.11	0.574
TSH, μ U/ml (0.4–4.0)	1.72 \pm 1.15	1.73 \pm 0.99	1.69 \pm 1.37	0.918
ftT3, pg/ml (1.8–4.8)	3.57 \pm 0.69	3.72 \pm 0.67	3.41 \pm 0.68	0.118
ftT4, ng/dl (0.8–1.8)	0.99 \pm 0.20	0.96 \pm 0.22	1.03 \pm 0.18	0.198
ACTH, pg/ml (<50)	40.42 \pm 21.80	44.10 \pm 25.22	36.43 \pm 17.01	0.218
Cortisol 8 am, μ g/dl (6.7–22.6)	19.50 \pm 9.27	17.41 \pm 9.47	21.75 \pm 8.69	0.098
Cortisol 4 pm, μ g/dl (<10.0)	11.81 \pm 5.01	11.09 \pm 3.95	12.68 \pm 6.06	0.312
PTH, pg/ml (8–80)	54.50 \pm 29.31	46.23 \pm 24.24	63.46 \pm 32.09	0.036
25(OH)D ₃ , ng/ml (11–70)	17.45 \pm 10.72	20.51 \pm 12.68	14.13 \pm 6.93	0.034
Folate, ng/ml (4.6–18.7)	5.45 \pm 1.80	5.39 \pm 1.61	5.52 \pm 2.02	0.799
Vitamin B12, pg/ml (191–663)	385.7 \pm 143.3	379.23 \pm 130.68	392.67 \pm 158.37	0.744

^aData are expressed as mean \pm SD.

with mGFR below the median value (i.e., 104.20 ml/min/1.73 m²) (R = 0.59, p = 0.0027; table 3).

Figure 1 shows Bland-Altman plots comparing mGFR with the three GFR estimation formulae. Bland-Altman analysis provided an unadjusted bias of –96.1 (–37.7 ml/min/1.73 m²) for Cockcroft-Gault, 11.3 ml/min/1.73 m² for MDRD, and 5.3 ml/min/1.73 m² for CKD-EPI. The standard deviation of the difference was equal to 57.5 (–34.2 ml/min/1.73 m²) for

Table 3. Correlation (Pearson's R) between mGFR and different estimation formulae in the study population as a whole (n = 50) and divided into above (n = 26) and below (n = 24) the median mGFR value (104.20 ml/min/1.73 m²)

	Intercept (t)	R	p value
All			
True/MDRD	8.278	0.450	0.0010
True/CKD/EPI	5.572	0.493	0.0003
True/Cockcroft	10.199	0.336	0.0171
Above median value			
True/MDRD	15.333	0.138	0.5005
True/CKD/EPI	12.279	0.039	0.8509
True/Cockcroft	21.163	0.166	0.4163
Below median value			
True/MDRD	8.974	0.477	0.0184
True/CKD/EPI	6.314	0.585	0.0027
True/Cockcroft	9.989	0.406	0.049

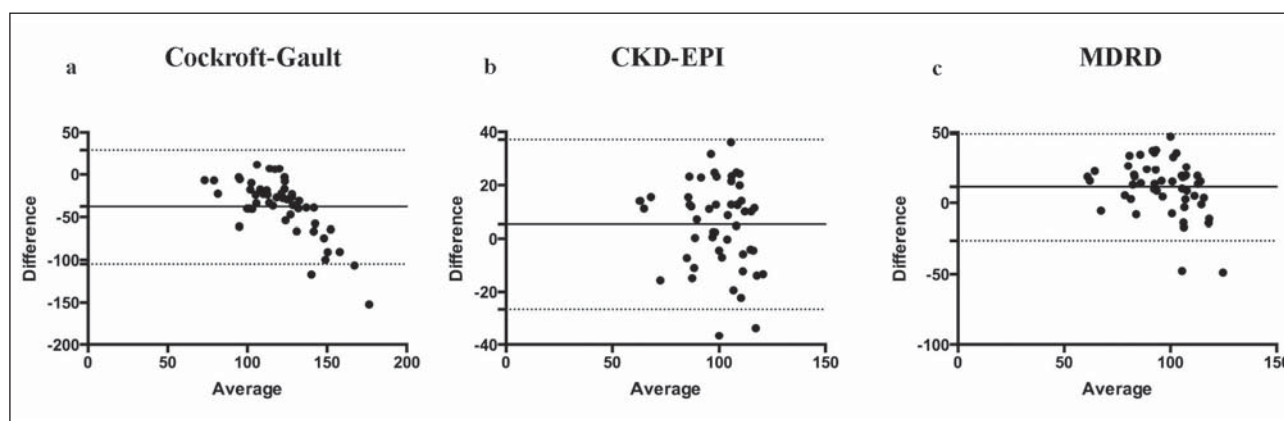


Fig. 1. Bland-Altman plots comparing mGFR with eGFR by **a** Cockcroft-Gault formula; **b** CKD-EPI formula; **c** MDRD formula. Black lines represent bias, while dotted lines represent ± 2 standard deviations.

Cockcroft-Gault, 19.2 ml/min/1.73 m² for MDRD, and 16.2 ml/min/1.73 m² for CKD-EPI. The difference between Cockcroft-Gault and mGFR was directly and significantly related to the average between the two measurements ($R = -0.73$, $p < 0.001$), indicating that overestimation by Cockcroft-Gault is greater for higher GFR.

Correlates of mGFR

mGFR was higher in men than in women (108 ± 13 vs. 98 ± 15 ml/min/1.73 m², $p = 0.015$) but was not related to age, BMI, serum creatinine, and brachial or central BP values. On the contrary, an interesting linear relationship emerged between mGFR and the total AUC ($R = 0.36$, $p = 0.009$) and IncrAUC of glucose ($R = 0.32$, $p = 0.02$). Furthermore, mGFR was correlated inversely with HDL cholesterol ($R = -0.29$, $p = 0.04$).

The hormonal profile of the study population is shown in table 2. Mean values were all within the normal range, even though with the noticeable tendency to low GH and folate and high cortisol levels. No relationships between hormonal pattern and mGFR emerged, except for an inverse linear relationship with PTH ($R = -0.319$, $p = 0.024$) and with h8 cortisol ($R = -0.31$, $p = 0.03$). A trend was observed with vitamin D. Comparing patients with mGFR above

Table 4. Main ultrasonographic parameters in obese individuals as a whole (n = 50) and divided into above (n = 26) and below (n = 24) the median mGFR value (104.20 ml/min/1.73 m²)^a

	All	Above	Below	p value
Brachial artery diameter, mm	4.26 ± 0.73	4.37 ± 0.95	4.14 ± 0.41	0.493
Renal longitudinal diameter, mm	11.634 ± 1.087	12.093 ± 1.108	11.176 ± 0.878	0.022
Aortic PWV, m/s	8.24 ± 1.22	8.20 ± 0.99	8.27 ± 1.50	0.892
FMD, %	4.54 ± 2.41	4.20 ± 2.30	4.89 ± 2.58	0.534
Augmentation index	18.09 ± 1.79	18.42 ± 8.63	17.73 ± 8.92	0.852
Renal RI	0.62 ± 0.05	0.64 ± 0.05	0.60 ± 0.04	0.029
DRIN	-5.70 ± 4.68	-5.23 ± 4.60	-6.22 ± 4.92	0.606

^aData are expressed as mean ± SD.

Table 5. Univariate and multivariate linear regression exploring variables associated with mGFR

Variable	Univariate linear regression		Multivariate linear regression (adjusted for age, sex, BMI)		
	R	p value	R ² full model	standardized coefficient	p value
BUN	-0.31	0.03	0.22	-0.31	0.03
IncrAUC	0.32	0.02	0.22	0.30	0.03
HDL cholesterol	-0.29	0.04	0.15	-0.11	0.45
PTH	-0.32	0.02	0.18	-0.24	0.10
h8 Cortisol	-0.31	0.03	0.15	-0.13	0.36
Renal diameter	0.57	0.0009	0.38	0.41	0.02
RPF	0.49	0.02	0.45	0.67	0.005

and below the median, the latter had significantly higher PTH levels (p = 0.036), and lower vitamin D3 (p = 0.034).

Table 4 shows the renal ultrasonographic measures in obese patients as a whole and according to the median mGFR value. Renal longitudinal diameter (mean value between right and left kidney) was significantly higher in patients above the median mGFR, with a linear correlation between the two parameters in the whole group (R = 0.57, p = 0.0009). Neither basal nor dynamic RI were related with mGFR, even if there was a small but significantly higher RI in those above the median mGFR value. Neither FMD nor PWV and wave reflection variables showed any relationship with mGFR.

Multiple regression analysis, aimed to evaluate independent determinants of mGFR in these morbidly obese individuals, was performed, testing each variable significantly associated with mGFR in the univariate analysis: IncrAUC, blood urea nitrogen (BUN), renal diameter, and RPF remained significantly associated with mGFR (table 5). In a further, fully adjusted model including IncrAUC, BUN, renal diameter and RPF together with age, sex and BMI, only renal diameter and RPF remained independently related with mGFR (full model R² = 0.67), accounting for 15% and 21% of mGFR variance, respectively.

Correlates of Measured Renal Plasma Flow

In the subset of 25 patients receiving the measurement of RPF (mean 609.14 ± 156.14 ml/min), it was significantly higher in those with mGFR ≥ 104.2 ml/min/1.73 m² (710.39 ±

176.59 vs. 555.13 ± 117.20 ml/min; $p = 0.02$). As expected, RPF was directly correlated with mGFR ($R = 0.49$, $p = 0.02$). RPF tended to be related with age ($R = -0.36$, $p = 0.09$), systolic BP ($R = 0.38$, $p = 0.08$) and creatinine ($R = -0.35$, $p = 0.09$). Interestingly, it was also related to GH ($R = -0.43$, $p = 0.04$), even though this correlation lost significance after adjustment for age, sex, and BMI ($p = 0.17$, standardized coefficient = -0.28 , full model $R^2 = 0.38$). Among vascular parameters, the only significant relation was between RPF and AIX ($R = 0.47$, $p = 0.03$), remaining significant even after adjustment for age, sex, and BMI ($p = 0.03$, standardized coefficient = 0.53 , full model $R^2 = 0.48$).

Mean filtration fraction was $17 \pm 4\%$, being similar in subjects above and below the median values. It correlated directly with age, systolic BP and renal diameter ($R = 0.74$; $p < 0.001$) and inversely with BMI, heart rate and, obviously, RPF ($R = -0.73$; $p < 0.0001$).

Discussion

The main novelties of the present study are: i) among formulae commonly used for estimating GFR, CKD-EPI shows the best performance respect to true GFR measurements in morbidly obese individuals with preserved renal function and no metabolic comorbidities; ii) glucose level increments during an OGTT are correlated to hyperfiltration; and iii) renal diameter and PTH are independent determinants of mGFR.

Previous studies have documented the unreliability of algorithms to estimate glomerular function in patients with obesity and diabetes, especially when suffering from CKD [25], but such comparisons in severely obese otherwise healthy individuals are scanty. We demonstrated that CKD-EPI has the best correlation with mGFR (data adjusted by surface area) with the lowest bias, as recently reported by Friedman et al. [26], and this was also true in the high range of GFR. Indeed, CKD-EPI was the only estimation formula with no increase in bias with increasing values of mGFR.

Noticeably, mean GFR value obtained in our patients is fully superimposable to that measured by Cr-EDTA technique in a subset of morbidly obese nondiabetic individuals [27]. Previous reports showed the largest influence of BMI on overestimation of GFR for Cockcroft-Gault formula, with less influence on MDRD and CKD-EPI [28]; which does not come as a surprise as body weight is a component of formula itself. Other differences reside in the fact that previous reports evaluated obese patients with various degrees of renal impairment [29] while, in our study population, none showed micro- or macroalbuminuria or had $GFR < 60$ ml/min/1.73 m².

We also addressed the thus far unsolved question whether estimation formulae can be adequately used for any threshold of BMI by comparing true and eGFR measures in severely obese people with preserved renal function, confirming a good accuracy also in people with BMI > 40 kg/m².

In type 2 diabetes and in pre-diabetic states, uncontrolled blood glucose levels are proportionately related to the severity of hyperfiltration, even after adjustment for confounding factors [30, 31]. The observation of total AUC and IncrAUC of glucose as clinical correlates of increased GFR in obese nondiabetic individuals points to a continuum of atherosclerotic risk factors, suggesting that fluctuations of glucose levels, which are more likely in the post-prandial phase, may act as determinant of glomerular hyperfiltration in obesity.

In the subset of individuals receiving also a measurement of RPF, we observed a strong linear correlation between GFR and RPF, with no apparent reduction of the filtration fraction in patients with so far fully preserved renal function, which was also confirmed by the fact that all were normoalbuminuric.

We did not find any relation between mGFR and BP or parameters of vascular function and structure in our study group. In contrast to previous cross-sectional studies in populations without CKD that did not find any correlation between eGFR and PWV [32, 33], in our population PWV and diastolic BP were significantly correlated with CKD-EPI ($R = -0.53$, $p = 0.01$ and $R = -0.33$, $p = 0.02$ respectively), but not with mGFR, suggesting that the CKD-EPI formula might depict vascular age beyond renal function, possibly due to the inclusion of age and sex in the formula. Commenting on the results of vascular variables, the relationship between Aix75, as a marker of wave reflection, and RPF deserves further attention. This finding suggests that in morbid obesity a hypertrophic renal vascular bed might enhance wave reflection, which could be associated with negative consequences on cardiovascular prognosis [34].

A novel approach of our study was to correlate true GFR and RPF with a complete hormonal pattern in severely obese patients. As expected [35], these individuals showed GH levels in the low normal range. Consequently there was no correlation with mGFR, suggesting as only a marked GH hypersecretion, like in acromegaly, may affect glomerular hyperfiltration [36]. Interestingly, the well-known inverse relationship between PTH and GFR as well as the relationship between vitamin D and GFR seem to be confirmed in morbidly obese individuals also in the presence of a fully preserved renal function, with a tendency to a slightly lower PTH and higher vitamin D levels in those with mGFR above the median value.

In conclusion, our study validates CKD-EPI as the best formula when compared to a true measure of GFR in severely obese people with preserved renal function and shows that glucose level increments during an OGTT are correlated to hyperfiltration. Its major limitations reside in the small size of the cohort and in its cross-sectional nature that does not allow to draw any conclusions regarding the potential long-term effect of even minor impairments of hormone patterns on long-term renal function.

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Disclosure Statement

The authors have no conflicts of interest to declare.

References

- 1 Kramer HJ, Saranathan A, Luke A, Durazo-Arvizu RA, Giuchan C, Hou S, Cooper R: Increasing body mass index and obesity in the incident ESRD population. *J Am Soc Nephrol* 2006;17:1453–1459.
- 2 Hsu CY, McCulloch CE, Iribarren C, Darbinian J, Go AS: Body mass index and risk for end-stage renal disease. *Ann Int Med* 2006;144:21–28.
- 3 Iseki K, Ikemiya Y, Kinjo K, Inoue T, Iseki C, Takishita S: Body mass index and the risk of development of end-stage renal disease in a screened cohort. *Kidney Int* 2004;65:1870–1876.
- 4 de Vinuesa SG, Goicoechea M, Kanter J, Puerta M, Cachofeiro V, Lahera V, Gomez-Campdera F, Luño J: Insulin resistance, inflammatory biomarkers, and adipokines in patients with chronic kidney disease: effects of angiotensin II blockade. *J Am Soc Nephrol* 2006;17(12 suppl 3):S206–S212.
- 5 Ritz E, Koleganova N, Piecha G: Is there an obesity-metabolic syndrome related glomerulopathy? *Curr Opin Nephrol Hypert* 2011;20:44–49.
- 6 Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, Coresh J, Gansevoort RT, Chronic Kidney Disease Prognosis Consortium: Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* 2010;375:2073–2081.

- 7 Van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, de Jong P, Gansevoort RT; Chronic Kidney Disease Prognosis Consortium: Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney Int* 2011;79:1341–1352.
- 8 Bello AK, Peters J, Wight J, El Nahas M: The Kidney Evaluation and Awareness Program in Sheffield (KEAPS): a community-based screening for microalbuminuria in a British population. *Nephron Clin Pract* 2010;116:c95–c103.
- 9 Tang J, Yan H, Zhuang S: Inflammation and oxidative stress in obesity-related glomerulopathy. *Int J Nephrol* 2012;608397.
- 10 Henegar JR, Bigler SA, Henegar LK, Tyagi SC, Hall JE: Functional and structural changes in the kidney in the early stages of obesity. *J Am Soc Nephrol* 2001;12:1211–1217.
- 11 Wuerzner G, Pruijm M, Maillard M, Bovet P, Renaud C, Burnier M, Bochud M: Marked association between obesity and glomerular hyperfiltration: a cross-sectional study in an African population. *Am J Kidney Dis* 2010;56:303–312.
- 12 de Vries AP, Ruggenenti P, Ruan XZ, Praga M, Cruzado JM, Bajema IM, D'Agati VD, Lamb HJ, Pongrac Barlovic D, Hojs R, Abbate M, Rodriquez R, Mogensen CE, Porrini E; ERA-EDTA Working Group Diabetes: Fatty kidney: emerging role of ectopic lipid in obesity-related renal disease. *Lancet Diabetes Endocrinol* 2014;2:417–426.
- 13 Jerums G, Premaratne E, Panagiotopoulos S, MacIsaac RJ: The clinical significance of hyperfiltration in diabetes. *Diabetologia* 2010;53:2093–2104.
- 14 Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration): A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604–612.
- 15 Murata K, Baumann NA, Saenger AK, Larson TS, Rule AD, Lieske JC: Relative performance of the MDRD and CKD-EPI equations for estimating glomerular filtration rate among patients with varied clinical presentations. *Clin J Am Soc Nephrol* 2011;6:1963–1972.
- 16 Bouquegneau A, Vidal-Petiot E, Vrtovnsnik F, Cavalier E, Rorive M, Krzesinski JM, Delanaye P, Flamant M: Modification of Diet in Renal Disease versus Chronic Kidney Disease Epidemiology Collaboration equation to estimate glomerular filtration rate in obese patients. *Nephrol Dial Transplant* 2013;28:122–130.
- 17 Abouchacra S, Chaaban A, Gebran N, Hussein Q, Ahmed M, Bernieh B, Torab F, Kayyal Y, Al Omary H, Nagelkerke N: GFR estimation in the morbidly obese pre- and postbariatric surgery: one size does not fit all. *Intern Urol Nephrol* 2013;45:157–162.
- 18 Redal-Baigorri B, Rasmussen K, Heaf JG: The use of absolute values improves performance of estimation formulae: a retrospective cross sectional study. *BioMed Central Nephrol* 2013;14:271.
- 19 Tublin ME, Bude RO, Platt JF: The resistive index in renal Doppler sonography: where do we stand? *Am J Roentgenology* 2003;180:885–892.
- 20 Kawai T, Kamide K, Onishi M, Yamamoto-Hanasaki H, Baba Y, Hongyo K, Shimaoka I, Tataru Y, Takeya Y, Ohishi M, Rakugi H: Usefulness of the resistive index in renal Doppler ultrasonography as an indicator of vascular damage in patients with risks of atherosclerosis. *Nephrol Dial Transplant* 2011;26:3256–3262.
- 21 Blaufox MD, Aurell M, Bubeck B, Fommei E, Piepsz A, Russell C, Taylor A, Thomsen HS, Volterrani D: Report of the Radionuclides in Nephrourology Committee on renal clearance. *J Nucl Med* 1996;37:1883–1890.
- 22 Ghiadoni L, Fatta F, Salvetti M, Cordiano C, Biggi A, Puato M, Di Monaco A, De Sisti L, Volpe M, Ambrosio G, Gemignani V, Muesan ML, Taddei S, Lanza GA, Cosentino F: Assessment of flow-mediated dilation reproducibility: a nationwide multicenter study. *J Hypertens* 2012;30:1399–1405.
- 23 Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FU, Protogerou AD, Schillaci G, Segers P, Vermeersch S, Weber T; Artery Society; European Society of Hypertension Working Group on Vascular Structure and Function; European Network for Noninvasive Investigation of Large Arteries: Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens* 2012;30:445–448.
- 24 Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–310.
- 25 Nair S, Mishra V, Hayden K, Lisboa PJ, Pandya B, Vinjamuri S, Hardy KJ, Wilding JP: The four-variable modification of diet in renal disease formula underestimates glomerular filtration rate in obese type 2 diabetic individuals with chronic kidney disease. *Diabetologia* 2011;54:1304–1307.
- 26 Friedman AN, Moe S, Fadel WF, Inman M, Mattar SG, Shihabi Z, Quinney SK: Predicting the glomerular filtration rate in bariatric surgery patients. *Am J Nephrol* 2014;39:8–15.
- 27 Belhatem N, Mohammedi K, Rouzet F, Matallah N, Al Baloshi A, Travert F, Velho G, Roussel R, Le Guludec D, Marre M, Hansel B: Impact of morbid obesity on the kidney function of patients with type 2 diabetes. *Diabetes Res Clin Pract* 2015;108:143–149.
- 28 Michels WM, Grootendorst DC, Verduijn M, Elliott EG, Dekker FW, Krediet RT; NECOSAD Study Group: Performance of the Cockcroft–Gault, MDRD, and new CKD-EPI formulas in relation to GFR, age, and body size. *Clin J Am Soc Nephrol* 2010;5:1003–1009.
- 29 Lemoine S, Guebre-Egziabher F, Sens F, Nguyen-Tu MS, Juillard L, Dubourg L, Hadj-Aissa A: Accuracy of GFR estimation in obese patients. *Clin J Am Soc Nephrol* 2014;9:720–727.
- 30 Okada R, Yasuda Y, Tsushita K, Wakai K, Hamajima N, Matsuo S: Glomerular hyperfiltration in prediabetes and prehypertension. *Nephrol Dial Transplant* 2012;27:1821–1825.

- 31 Melsom T, Mathisen UD, Ingebrechtsen OC, Jenssen TG, Njølstad I, Solbu MD, Toft I, Eriksen BO: Impaired fasting glucose is associated with renal hyperfiltration in the general population. *Diabetes Care* 2011;34:1546–1551.
- 32 Upadhyay A, Hwang SJ, Mitchell GF, Vasan RS, Vita JA, Stantchev PI, Meigs JB, Larson MG, Levy D, Benjamin EJ, Fox CS: Arterial stiffness in mild-to-moderate CKD. *J Am Soc Nephrol* 2009;20:2044–2053.
- 33 Bruno RM, Penno G, Daniele G, Pucci L, Lucchesi D, Stea F, Landini L, Cartoni G, Taddei S, Ghiadoni L, Del Prato S: Type 2 diabetes mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction. *Diabetologia* 2012;55:1847–1855.
- 34 Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C: Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J* 2010;31:1865–1871.
- 35 Maccario M, Tassone F, Grottoli S, Rossetto R, Gauna C, Ghigo E: Neuroendocrine and metabolic determinants of the adaptation of GH/IGF-I axis to obesity. *Ann Endocrinol (Paris)* 2002;63:140–144.
- 36 Grunenwald S, Tack I, Chauveau D, Bennet A, Caron P: Impact of growth hormone hypersecretion on the adult human kidney. *Ann Endocrinol (Paris)* 2011;72:485–495.